ARTIFICIAL INCUBATION OF SOME NON-GALLIFORM EGGS

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Although there is an extensive literature on artifical incubation of eggs of domestic chicksns and some other galliform birds, relatively little has been published concerning artificial incubation in other birds. Data gained from artificial incubation of wild birds' eggs may have great importance in several phases of ornithology. For example, might not the tolerance of the embryo for humidity or extremes of temperature be an important limiting factor in the breeding distribution of a given species? Also, why is there great variation in length of incubation period among some closely related species? By controlling and varying the conditions of temperature and moisture in the incubation chamber, many such questions could be answered at least partially. Uniformly-incubated eggs are also useful material in cytogenetic studies and studies in comparative embryology.

During the summer of 1954 I tried to determine what problems were involved in a study on artificial incubation, and gathered the few data presented, with a minimum of crude equipment and in the spare time from another project.

Methods

As an incubator I used an asbestos-walled drying oven which measured 10 × 10 × 12 inches. I fitted it with a cartridge-type thermoregulator with a range from minus 100 to 400 degrees F. In the bottom of the oven were metal coils which provided the heat source when connected to standard 110-volt electric circuit. The incubator had two ¼-inch mesh hardware cloth shelves about equidistantly spaced in the chamber. On the bottom shelf (nearest the heating coils) I placed a shallow 9 × 9-inch cake pan which I kept filled with water as a source of moisture for the eggs. The top shelf I used as an egg tray. No fan to stir the air in the incubator was used; this probably would have greatly enhanced temperature constancy.

The question of the temperature to use in incubation was difficult to decide. Baldwin and Kendeigh's work on bird temperature (1932), and Huggins' (1941) work on egg temperatures guided me to a certain extent. Kendeigh (1940:507) stated that "Romanoff (1934) found an increased rate of growth and percentage hatch of pheasant and Bob-white embryos up to 38.3° or 38.9° C. but a distinct retardation at 40.6° C." However, since I planned to work largely with Bell's Vireos (*Vireo belli*), a common species in central Oklahoma whose nests are easily found, I tried to learn about incubation temperatures in this species. This I did by inserting a maximum-registering thermometer through the wall of a vireo nest so that the bulb rested in the midst of the eggs. In this manner I checked a few different nests by leaving the thermometer in place over night and taking a reading on the following morning. Maximum temperatures obtained in this way on different nests were 99°, 100°, and 102° F. Outside temperatures during these tests in early June ranged from 70° to 80° F., both mornings and evenings.

I finally planned to run the incubator at about 38° C. (app. 100° F.), but the best adjustment I could make produced a range from 36.5° C. (98° F.) to 39° C. (102° F.) By keeping a maximum-registering thermometer on the egg tray I knew the maximum temperature to which any egg was exposed. Shortly after I introduced the first eggs into the incubator, its temperature range increased to a usual high of 40° C. (104° F.) from a low of 36.5° C. (98° F.). This was the usual fluctuation throughout the study, though a few times the temperature fell to 35° C. (95° F.) and rose to 41° C. (106° F). On one occasion each, the temperature rose to 42° C. (108° F.), 44.5° C. (112° F.), and 49° C. (120° F.). These 'accidents' helped show the extent of heat tolerance in Brown-headed Cowbird (*Molothrus ater*) and Cardinal (*Richmondena cardinalis*) embryos.

I had no apparatus for recording temperature or humidity, so the figures represent only my recordings. I noted temperature and relative humidity at least four times each 24 hours, and once made 19 recordings in one 17-hour period. Readings of temperature were taken from one Centigrade (–20° to 110°), and one Fahrenheit (–30° to 120°) thermometer. These were inserted in corks in the top of the incubator so that their bulbs were near the egg tray, but the bulk of the columns could be read outside the incubator. These thermometers and the maximum registering thermometers used showed good correlation in reading. I had less control of humidity in this small chamber. Relative humidity readings were taken with an aspirator-type psychrometer, by placing the end of the aspirator against a hole (which was ordinarily covered) in the top of the incubator.

The average of the total (160) recorded incubation temperatures for the entire study period (June 8 to July 11) was 37.5° C. (100° F.) The average of all humidity readings was 59.7 per cent (minimum, 40 per cent; maximum 74). Once I had put eggs into the incubator I made no further adjustment, but merely recorded the conditions that existed. Eggs were exposed to room conditions three times a day for less than one minute, at about 4:00 a.m., 12:00 p.m., and 9:00 p.m., when I turned each egg by hand. All eggs were collected in the vicinity of Cogar, Caddo County, Oklahoma, and were taken directly from nest to incubator. Each egg was numbered and weighed on a centigram balance before it was put into the incubator. The time and date of entry were recorded. I also weighed some partially-incubated eggs, and in a few cases obtained the weights of dry, freshly-hatched birds.

Results

Eggs of the Mourning Dove (*Zenaidura macroura*), Yellow-billed Cuckoo (*Coccyzus americanus*), Bell's Vireo, Brown-headed Cowbird, Cardinal, and Painted Bunting (*Passerina ciris*) were hatched successfully in the incubator, though only the ages of the vireo eggs and two of the dove eggs were known.

Table 1 summarizes part of the data on five species. The period indicated under pipping and hatching is not the true incubation period, of course, but only the period of artificial incubation.

Table 1

Partial Summary of Artificial Incubation Data

Species	Egg No.	Days and hours incubation: pipped	Days and hours incubation: hatched	Incubation temperature (1): Average		Minimum		Maximum		Relative Humidity: Avg.	Min.	Max.
Zenaidura macroura	1	11- 1	11- 5	37	(98.5)	35	(95)	41	(106)	50	41	60%
	2	12-16	13-20	37		35		41		50	41	60%
	3	13- 0	14-13	37		35		41		50	41	60%
Coccyzus americanus	1	9- 0	9- 4	38.5	(101)	35		42.5	(108)	60	40	74%
	2	7-14	8- 0	37.5	(99.5)	35		41	(106)	54	43	66%
Molothrus ater	1		8- 7	38.5		36.5	(98)	49	(120)	58	40	74%
	2		1- 0	38	(100.5)	36.5		42	(108)	60	52	68%
	3	9-12		38.5		35		44.5	(112)	60	41	74%
	4		9-12	38.5		35		44.5		60	41	74%
Richmondena cardinalis	1		7- 0	38.5		36		48.9	(120)	58	40	70%
	2	7-10	7-18	38.5		36		48.9		60	40	74%
Passerina ciris	(2 eggs)		1-16	39	(102)	36.5		41		63	48	68%

1. Degrees Centigrade, followed by Fahrenheit equivalent (in parentheses).

Some of the species warrant more detailed discussion and tabulation. They will be considered in order of the amount of data.

Bell's Vireo Eggs

Twelve Bell's Vireo eggs (three clutches) were incubated; of these nine piped but only five hatched (three of one clutch, one of each of the others). Of the four which pipped but did not hatch, all apparently were normally formed, and only one had not absorbed all of the external yolk. Three eggs were not pipped. I broke one of these in handling, perforated another with a minute hole, and the third, the last egg laid in clutch 1, showed no obvious development after 16 days of incubation. Data on this species are summarized in Tables 2 and 3.

Incubation began with the second egg in clutch 1, but I believe (for reasons given below) that it began with the third egg in clutches 2 and 3.

The variation in weight of eggs from different clutches is interesting and reflected a difference in size which was conspicuous, although I made no measurements. Note difference in weights of eggs *2, 3* and *2a, 3a* at same age (Table 3), and the progressive increase in egg weight from the first to the last egg in the clutch, in all three clutches. Note also that the three eggs of clutch 3 which hatched were also the smallest (lightest). Egg *4,* which showed no development, lost weight at the same rate as developing eggs. Egg *1a,* which was minutely perforated, lost about half its weight in 11 days. In this period weight loss of developing eggs averaged 5.4 per cent of initial weight in clutch 1 (heavier), and about 4 per cent in clutch 2 (lighter).

Table 2

Incubation Data for those Bell's Vireo Eggs in which Development was Completed

(Time counted to perforation of shell in pipping. Eggs checked at least every four hours during the day.)

Clutch No.	Egg No.	Incubation by birds: days	Incubation by birds: hours	Incubator Total: days	Incubator Total: hours	Total Incubation	Incubation Temperature (1): Average		Min.		Max.		Relative Humidity: Avg.	Relative Humidity: Min.
1	2	2	12	12	12	15 days 0 hrs.	37.8	(100)	35	(95)	42.5	(108)	41	62
	3	0	14	14	21	14 days 21 hrs.	38	(100)	35		44.5	(112)	41	62
2	2a	1	10	13	12	14 days 22 hrs.	37.8		35		42.5		41	62
	3a	1	10	13	12	14 days 22 hrs.	37.8		35		42.5		41	62
	4a	0	10	14	13	14 days 23 hrs.	38.3		35		42.5		41	62
3	1b	6	6	8	18	15 days 0 hrs.	37.2		35		41	(106)		
	2b	6	6	8	20	15 days 2 hrs.	37.2		35		41			
	3b	6	6	9	2	15 days 8 hrs.	37.2		35		41			

The incubation period in each case was within a few hours of 15 days, that is, to the time of pipping through the shell. The data are presented in this way because more birds pipped than hatched, and those which hatched successfully did so within about six hours of breaking the shell. Pitelka and Koestner (1942: 99) give the incubation period for this species as 14 days. The discrepancy between this figure and mine is not surprising, but it is interesting that the artificial incubation period did not change even when natural incubation was performed on four eggs for over a third of the period. The incubation period was especially uniform within the clutch. If the period may vary from clutch to clutch (it seemed to vary slightly even under similar incubation conditions), this is of interest because the shortest possible incubation period that produces sound hatchlings obviously has the best survival value. This suggests a reason why there is much variation in incubation period among closely related species (as in *Vireo*). It is probably an important factor in 'success', i.e., broadness of distributional range or density of population among birds.

Three of the four eggs which were incubated by the parent for the longest

TABLE 3

WEIGHT RELATIONSHIP OF BELL'S VIREO EGGS

Egg	Date laid*	Date entry incubator	Weight (grams)	Second weighing date	Second weighing grams	Date and hour pipped	Weight hatched bird	Remarks
1	June 12	June 15	1.67					broken June 23
2	June 13	June 15	1.79	June 26	1.70	June 28 5:00 a.m.	1.50	hatched June 28 by 4 p.m.
3	June 14	June 14	1.81	June 26	1.70	June 29 4:00 a.m.		dead in egg June 29
4	June 15	June 15	1.82	June 26	1.73			no development after 16 days incubation
1a	June 12	June 15	1.31	June 26	.72			minutely perforated on June 19
2a	June 13	June 15	1.43	June 26	1.36	June 29 4:00 a.m.		helped from shell at pipping
3a	June 14	June 15	1.52	June 26	1.47	June 29 4:00 a.m.		dead in egg June 29
4a	June 15	June 15	1.63	June 26	1.57	June 30 5:00 a.m.		helped from shell at pipping; died shortly
1b	June 21	June 29	1.29					hatched July 8 by 11:00 a.m.
2b	June 22	June 29	1.24					hatched July 8 12:00 p.m.
3b	June 23	June 29	1.25					hatched July 8 4:00 p.m.
4b	June 24	June 29	1.35					dead in egg July 9

*All eggs laid in early morning

period hatched successfully, as opposed to two of five (that pipped) that were largely artificially incubated. However, egg *4a* might have succeeded without my interference. If temperature were a factor in the apparent reduced vitality of the birds which pipped but did not hatch, then a lower temperature would probably have favored them, in view of Romanoff's findings (see above), and the fact that Huggins (*op. cit.*) found egg temperatures of wild birds to average several degrees lower than the average temperature in my incubator. I would expect the temperature of an egg in my incubator to be at least as high as the average incubation temperature since the 'attentive' period is virtually uninterrupted.

Romanoff (1949) discussed the periods and causes of mortality in avian embryos. He pointed out several causes of death in the late critical period and "that at the end of the developmental period the cumulative effect of all unfavorable conditions may be felt." I noted that I could often hear the embryo tapping on the inside of the shell a full day before any noticeable mark could be seen. Generally the first marks were mere bulges which appeared several hours before the shell actually was perforated. The fact that three birds which died before hatching had actually perforated the shell in a place or two, made me wonder if thinning of the shell by wear in the nest is not a factor in hatching success. Eggs from my incubator were not worn much by my system of turning since I picked the egg up without rubbing it against any surface. I seriously doubt that thickness of the shell is an important factor unless the embryo is weak anyway.

Hanson (1954) has recently shown that the increasing opacity of the egg with development of the embryo has utility as a fairly accurate indicator of age in incubated eggs and in determining with which egg of the clutch incubation began, especially in whitish or non-opaque eggs. On the sixth day of natural incubation I could see by candling the eggs in sunlight that eggs *1b*, *2b*, and *3b* were equally opaque to a degree that the vascular net could just be detected. Egg *4b*, on the other hand, was much less opaque and I could see the vascular net very clearly. The first three eggs also pipped and hatched together, while *4b* pipped considerably later. After the embryo reaches a certain stage, the degree of opaqueness does not change, but until that time the feature has a practical application. (See also the table on Mourning Dove development.)

Mourning Dove Eggs

The two eggs of *Zenaidura* clutch 1 were found on June 23, and were placed in the incubator at 12:00 noon when egg *1* weighed 5.68 grams, and *2* weighed 6.24 grams. Candling in sunlight showed a distinct embryo and vascular net in egg *1* while egg *2* showed no obvious development. The later egg was also heavier in the other clutch (see Table 4). On June 26, egg *1* weighed 5.55 grams and *2* weighed 6.24 grams. Other data on these eggs are summarized in Table 1.

On June 25 I found the first egg of clutch 2. It was being incubated in mid-morning. The second egg was laid on the morning of June 26, and I placed both eggs in the incubator on that date at 11:00 a.m., directly from the nest. Table 4 summarizes apparent development observed by candling, and other data on these eggs.

TABLE 4

DEVELOPMENT OF TWO MOURNING DOVE EGGS AS OBSERVED BY CANDLING

Date	time	Egg 3	Egg 4
June 26	11:00 a.m.	6.53 grams no obvious development	7.16 grams laid by 10:00 a.m. no obvious development
June 27	9:00 p.m.	no obvious development	no obvious development
June 28	5:00 a.m.	definite vascular net over one-fourth egg area (surface)	no obvious development
	9:00 p.m.	vascular net spread over one-half egg area	clouded in central band but no definite structure apparent
June 29	12:00 p.m.		no change apparent
	9:00 p.m.		small but distinct embryo and vascular net that covers about one-third of egg area
July 9	10:00 a.m.	egg shell bulged slightly but not pipped thru	
July 10	9:00 p.m.	hatched between these hours	
July 11	6:00 a.m.	dry at this time	
July 14	p.m.		embryo pecking inside egg shell shell not visibly marked
July 15	p.m.		egg opened; embryo dead, fully developed but some yolk not absorbed.

Three days and at least 11 hours were required for egg *4* to reach the stage of development that egg *3* had reached at 5:00 a.m. on June 28. Assuming a similar rate of development in both, the incubation period of egg *3*, largely under artificial conditions (see Table 1), was between 14 days, 16 hours, and 15 days. Fifteen days is the period in nature, according to Bent (1932:405). The average incubation temperature for the dove eggs was 37° C. (98.6° F.), and Huggins (*op. cit.*:150) found the average temperature to be 36.2° C. (97° F.) in the dove eggs he checked in nature. The correlation of incubation period in nature and in my incubator is very close for this species. Examine the comparable situation with the vireos. Huggins' (*loc. cit.*) figure for average egg temperature for three Red-eyed Vireo (*Vireo olivaceus*) eggs was 32.7° C. (91° F.). If that for Bell's Vireo is comparably low (in nature), then high incubation temperature was a likely factor in the increased incubation period (15 days as opposed to 14 in nature) of vireo eggs under artifical conditions, as well as in the increased mortality of embryos.

Brown-headed Cowbird Eggs

Data on *Molothrus* eggs are partially summarized in Tables 1 and 5. Three of five cowbird eggs(nos. *1*, *2*, and *4*) hatched in the incubator. Egg no. *3* pipped but did not hatch. When I opened this egg the following day I found that the skin of the head adhered to the shell membrane. Otherwise the embryo appeared normal. The history of these eggs deserves brief description. Egg *1* had been incubated by vireos at least one day, and was subsequently deserted for at least one day. During the 24-hour inattentive period the local weather station recorded temperature extremes of 65° to 90° F., which was probably the minimum range to which the egg was exposed. In addition it was once exposed to a temperature of 49° C. (120° F.) in the incubator, yet it hatched within nine days of these exposures. Eggs *3* and *4* were incubated by vireo hosts for at least three days before being deserted. They were unattended at least 15 hours before I placed them in the incubator. During this period of inattention they were exposed for a full night, during which there was a brief cool shower, and temperatures as given by the local weather station ranged between 61° and 87° F. On the fourth day of incubation these eggs were exposed to a temperature of 45° C. (112° F.) ; even so egg *3* pipped, and egg *4* hatched after 9½ days of incubation.

Table 5

Summary of Data on Weight of Cowbird Eggs and Young

Egg no.	Date entry incubator	grams	2nd date weighed	grams	Date pipped or hatched	Wt. of hatched bird (grams)
1	9 June	2.89	14 June	2.86	17 June	2.32
2	14 June	3.01			15 June	2.54
3	15 June	2.68			25 June	
4	15 June	2.88			25 June	2.30
5	19 June	2.85	26 June	2.72	(anomalous embryo died in shell)	

This apparent broad temperature tolerance of cowbird embryos would seem definitely to be advantageous in view of the breeding habits of this species. It led me to experiment by keeping three cowbird eggs on a tray in my work room where the daily temperature (in July) rose to about 102° F. and fell at night to between 70° and 85° F. I turned them as I did the incubator eggs, but otherwise they were subject to the environment of the room. My relative humidity records of the room for this period have been lost, but figures from the weather station indicate a range from about 30 to 68 per cent. My records for June in the incubation room average about 60 per cent, but I am sure that the July average was considerably lower. The three eggs lost an average of 8.3 per cent of their initial weights in nine days, and none showed development at the end of that time.

Cowbird egg *2* came from a Painted Bunting nest, and was introduced into the incubator at the same time as the host eggs. The parasite hatched about 16 hours in advance of the host young, and all hatched within two days of entry into the incubator.

In connection with study of artificially-incubated eggs, a fascinating side project suggests itself. Observation of hatchlings introduced to foster parents offers interesting possibilities. Rather than destroy some of the hatchlings, I offered them to foster parents. I put two Painted Buntings (eggs *1* and *2*) and a Cardinal (egg *1*), which hatched about the same time, in a Painted Bunting nest after taking its contents of eggs. All of these birds fared well and fledged. There was no apparent conflict between young and adults, and the adult buntings did not seem unduly strained in keeping the entire brood fed. A Cardinal (egg *2*) which I turned over to a pair of Bell's Vireos did poorly after the third day. It seemed actually to be starving and left the nest after only six days. I doubt that it survived. This Cardinal was placed in the nest of the parents of vireo clutch 1 within a few hours of the laying of the fourth egg of the clutch. The vireos stayed within a few feet and saw me remove their eggs and leave the hatchling, yet they accepted the condition without hesitation and were bringing green larvae to the Cardinal in less than one minute. I placed egg shells in the nest with each young bird, and invariably these were removed immediately. Vireos from eggs *2* and *2a* were placed in a Bell's Vireo nest on June 29. They fledged successfully on July 10 and 11 respectively. The time required for a complete cycle of these two eggs from laying through artificial incubation and fledging was 27 days. I mention these cases because they seem to present a clear insight into certain facets of bird behavior, and I believe such experiments, if well planned, could have scientific value.

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